526 Rec'd PCT/PTO 07 JUL 2000

FORM PTO-1390 (REV 12-29-99) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	A-69293/AJT
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	09/600021

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US98/11958	09 June 1998	09 June 1997

TITLE OF INVENTION
Method and Apparatus for Detecting Microparticles in Fluid Samples

APPLICANT(S) FOR DO/EO/US
Philippe Goix

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [ ] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [X] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    International Preliminary Examination Report (PCT Rule 71.1)
    International Search Report (PCT Rule 44.1)
    Power of Attorney by Inventor
    Revocation and Substitution of Power of Attorney by Guava
    Small Entity Declaration
    Petition to Revive Unintentionally Abandoned Patent Application

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/600021	PCT/US98/11958	A-69293/AJT

	CALCULATIONS PTO USE ONLY
17. [x] The following fees are submitted:	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$690.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 96.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [x] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	22 - 20 =	2	X **$18.00**	$ 36.00
Independent claims	1 - 3 =	0	X **$78.00**	$ 0
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$260.00**	$ 0
TOTAL OF ABOVE CALCULATIONS =				$ 262.00
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 131.00
SUBTOTAL =				$ 131.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0
TOTAL NATIONAL FEE =				$131.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00
TOTAL FEES ENCLOSED =				$171.00
			Amount to be refunded:	$
			charged:	$171.00

a. [x] A check in the amount of $ 171.00 to cover the above fees is enclosed. Petition to Revive Unintentionally Abandoned Application - $605.00

b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [x] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1300. A duplicate copy of this sheet is enclosed. (order #A-69293/AJT)

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO.

Aldo J. Test
FLEHR HOHBACH TEST ALBRITTON & HERBERT LLP
Suite 3400, 4 Embarcadero Center
San Francisco, CA 94111-4187

SIGNATURE

Aldo J. Test
NAME

18,048
REGISTRATION NUMBER

PTO/SB/10 (1-99)
Approved for use through 09/30/2000. OMB 0651-0031
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(c))--SMALL BUSINESS CONCERN

Docket Number (Optional)
A-69293/ajt

Applicant, Patentee, or Identifier: Philippe Goix
Application or Patent No.: New U.S. from PCT/US98/11958
Filed or Issued: International Application filed 09 June 1998
Title: Method and Apparatus for Detecting Microparticles and Sub-Micron Particles

RECEIVED
24 JUL 2000
Legal Staff
International Division

I hereby state that I am
- ☐ the owner of the small business concern identified below:
- ☒ an official of the small business concern empowered to act on behalf of the concern identified below.

NAME OF SMALL BUSINESS CONCERN Guava Technologies, Inc.

ADDRESS OF SMALL BUSINESS CONCERN 863 Mitten Road, Building C
Burlingame, CA 94010-1303

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 13 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office. Questions related to size standards for a small business concern may be directed to: Small Business Administration, Size Standards Staff, 409 Third Street, SW, Washington, DC 20416.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

- ☒ the specification filed herewith with title as listed above.
- ☐ the application identified above.
- ☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:
- ☒ no such person, concern, or organization exists.
- ☐ each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING Philippe Goix

TITLE OF PERSON IF OTHER THAN OWNER President

ADDRESS OF PERSON SIGNING 863 Mitten Rd., Bldg. C., Burlingame, CA 94010

SIGNATURE _____ DATE 6-29-2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Philippe J. Goix

based on Int'l App. No. PCT/US98/11958
International Filing Date: 09 June 1998

For: *Method and Apparatus for Detecting Microparticles in Fluid Samples*

PRELIMINARY AMENDMENT

Date: July 7, 2000

"EXPRESS MAIL" Mailing Label Number EL484658311US
Date of Deposit: July 7, 2000
I hereby certify that this document or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service Under 37 CFR 1.10 on the date indicated above and is addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

SIGNED _____
Kari Bateman

Commissioner of Patents and Trademarks
Washington D.C. 20231

Sir:

This is a Preliminary Amendment being filed in the above-referenced application. Please amend the application as follows:

IN THE CLAIMS:

Please amend the claims as follows. All pending claims, whether or not amended, are presented below for the Examiner's convenience.

1. (amended) A device for detecting a microparticle in a fluid, the microparticle being tagged with a fluorescent substance, the fluorescent substance emitting fluorescent light when exposed to electromagnetic radiation, the device comprising:

a capillary [chamber] tube;

a fluid delivery system coupled to the capillary [chamber] tube, the fluid delivery system capable of [introducing] causing the microparticle and the fluid [into] to flow through the capillary [chamber] tube;

a source of electromagnetic radiation [positioned] for projecting a beam of electromagnetic radiation of predetermined size through to capillary tube in proximity to the capillary [chamber]

tube to expose the fluorescent substance in a volume of the fluid defined by the beam size or the beam size and the interior of the capillary tube to electromagnetic radiation; and

a detection device configured and disposed to [measure] detect fluorescent light emitted from the fluorescent substance at an angle to the direction of the beam when the microparticle is in the capillary [chamber] volume and provide an output signal.

2. (unchanged) The device of claim 1, wherein the microparticle is a microorganism.

3. (unchanged) The device of claim 1, wherein the microparticle is a bacterium, virus, or parasite.

4. (unchanged) The device of claim 1, wherein the microparticle is a CD4 cell.

5. (unchanged) The device of claim 1, wherein the microparticle is a fluosphere.

6. (unchanged) The device of claim 5, wherein the fluosphere has been ingested by a filtro-feeder.

7. (unchanged) The device of claim 6, wherein the filtro-feeder has a feeding rate sensitive to a toxicant level in the fluid sample.

8. (unchanged) The device of claim 1, wherein the fluorescent substance is a dye-conjugated antibody.

9. (unchanged) The device of claim 1, wherein the fluorescent substance is a DNA stain.

10. (unchanged) The device of claim 1, wherein the fluorescent substance has a magnetic charge.

11. (unchanged) The device of claim 10, further comprising:

a magnetic element positioned in a surrounding relationship to the capillary, the magnetic element having a magnetic charge which repels the fluorescent substance.

12. (unchanged) The device of claim 1, wherein the fluid delivery system is a syringe coupled to a syringe pump.

13. (unchanged) The device of claim 1, wherein the fluid delivery system is a peristaltic pump.

14. (unchanged) The device of claim 1, wherein the source of electromagnetic radiation is at least one laser.

15. (unchanged) The device of claim 1, wherein the detection device is an array of detectors.

16. (amended) The device of claim 1, including a photodetector for collecting fluorescent light from the capillary volume and providing an output signal.

[A device for detecting a fluorescent substance tagged to a microparticle, comprising:

a single capillary flow carrier system for transporting the microparticle past a selected location;

a source of electromagnetic radiation for irradiating the substance tagged to the microparticle; and

a detection system for measuring fluorescent light emitted from the substance at the selected location.]

17. (amended) The device of claim [16] 1, including means for receiving the output signal from the detection device, processing the signal and providing an output representative of the amount of the fluorescent substance [wherein the source of electromagnetic radiation comprises a source of light].

18. (amended) The device of [claim] 16 or 17, including means for receiving and processing the output from the photodetector to analyze the Mie scattering peaks [wherein the source of light comprises a laser].

19. (amended) The device of claim [16] 1, wherein the source of electromagnetic radiation comprises a source of light [a plurality of microparticles are individually transported past the selected location at a substantially uniform velocity].

20. (amended) The device of claim [16] 1, wherein the source of light comprises a laser [microparticle is an organism].

21. (amended) The device of claim [16] 1, wherein a plurality of microparticles are individually transported past the selected location at a substantially uniform velocity [the microparticle is a fluosphere].

Cancel claim 22.

Cancel claim 23.

24. (amended) The device of claim [23] 7, wherein the fluorescent substance is ingested by the filtro-feeder, and exposure of the filtro-feeder to the toxic substance affects the rate of ingestion of the fluorescent substance by the filtro-feeder.

25. (amended) The device of claim [24] 8, further comprising means for calculating the ingestion rate as a function of the amount of fluorescent light emitted from the fluorescent substance at the selected location.

Cancel claim 26.

Cancel claim 27.

Cancel claim 28.

Cancel claim 29.

Cancel claim 30.

Cancel claim 31.

Cancel claim 32.

Cancel claim 33.

Cancel claim 34.

Cancel claim 35.

Cancel claim 36.

Cancel claim 37.

REMARKS

The claims have been amended to better define applicant's invention and distinguish it over the prior art.

The Commissioner is hereby authorized to charge any fee which is determined to be due in connection with this communication to our Deposit Account No. 06-1300 (Order No. A-69293/AJT).

Respectfully submitted,

By: _____
Aldo J. Test, No. 18,048

FLEHR HOHBACH TEST ALBRITTON &HERBERT LLP
Suite 3400, 4 Embarcadero Center
San Francisco, CA 94111-4187
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Philippe J. Goix based on Int'l App. No. PCT/US98/11958 International Filing Date: 09 June 1998	**PRELIMINARY AMENDMENT**
For: *Method and Apparatus for Detecting Microparticles in Fluid Samples*	Date: July 7, 2000

"EXPRESS MAIL" Mailing Label Number EL484658311US
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I hereby certify that this document or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service Under 37 CFR 1.10 on the date indicated above and is addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

SIGNED _____
Kari Bateman

Commissioner of Patents and Trademarks
Washington D.C. 20231

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a fluid delivery system coupled to the capillary [chamber] tube, the fluid delivery system capable of [introducing] causing the microparticle and the fluid [into] to flow through the capillary [chamber] tube;

a source of electromagnetic radiation [positioned] for projecting a beam of electromagnetic radiation of predetermined size through to capillary tube in proximity to the capillary [chamber]

tube to expose the fluorescent substance in a volume of the fluid defined by the beam size or the beam size and the interior of the capillary tube to electromagnetic radiation; and

a detection device configured and disposed to [measure] detect fluorescent light emitted from the fluorescent substance at an angle to the direction of the beam when the microparticle is in the capillary [chamber] volume and provide an output signal.

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3. (unchanged) The device of claim 1, wherein the microparticle is a bacterium, virus, or parasite.

4. (unchanged) The device of claim 1, wherein the microparticle is a CD4 cell.

5. (unchanged) The device of claim 1, wherein the microparticle is a fluosphere.

6. (unchanged) The device of claim 5, wherein the fluosphere has been ingested by a filtro-feeder.

7. (unchanged) The device of claim 6, wherein the filtro-feeder has a feeding rate sensitive to a toxicant level in the fluid sample.

8. (unchanged) The device of claim 1, wherein the fluorescent substance is a dye-conjugated antibody.

9. (unchanged) The device of claim 1, wherein the fluorescent substance is a DNA stain.

10. (unchanged) The device of claim 1, wherein the fluorescent substance has a magnetic charge.

11. (unchanged) The device of claim 10, further comprising:

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14. (unchanged) The device of claim 1, wherein the source of electromagnetic radiation is at least one laser.

15. (unchanged) The device of claim 1, wherein the detection device is an array of detectors.

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[A device for detecting a fluorescent substance tagged to a microparticle, comprising:

a single capillary flow carrier system for transporting the microparticle past a selected location;

a source of electromagnetic radiation for irradiating the substance tagged to the microparticle; and

a detection system for measuring fluorescent light emitted from the substance at the selected location.]

17. (amended) The device of claim [16] 1, including means for receiving the output signal from the detection device, processing the signal and providing an output representative of the amount of the fluorescent substance [wherein the source of electromagnetic radiation comprises a source of light].

18. (amended) The device of [claim] 16 or 17, including means for receiving and processing the output from the photodetector to analyze the Mie scattering peaks [wherein the source of light comprises a laser].

19. (amended) The device of claim [16] 1, wherein the source of electromagnetic radiation comprises a source of light [a plurality of microparticles are individually transported past the selected location at a substantially uniform velocity].

20. (amended) The device of claim [16] 1, wherein the source of light comprises a laser [microparticle is an organism].

21. (amended) The device of claim [16] 1, wherein a plurality of microparticles are individually transported past the selected location at a substantially uniform velocity [the microparticle is a fluosphere].

Cancel claim 22.

Cancel claim 23.

24. (amended) The device of claim [23] 7, wherein the fluorescent substance is ingested by the filtro-feeder, and exposure of the filtro-feeder to the toxic substance affects the rate of ingestion of the fluorescent substance by the filtro-feeder.

25. (amended) The device of claim [24] 8, further comprising means for calculating the ingestion rate as a function of the amount of fluorescent light emitted from the fluorescent substance at the selected location.

Cancel claim 26.

Cancel claim 27.

Cancel claim 28.

Cancel claim 29.

Cancel claim 30.

Cancel claim 31.

Cancel claim 32.

Cancel claim 33.

Cancel claim 34.

Cancel claim 35.

Cancel claim 36.

Cancel claim 37.

REMARKS

The claims have been amended to better define applicant's invention and distinguish it over the prior art.

The Commissioner is hereby authorized to charge any fee which is determined to be due in connection with this communication to our Deposit Account No. 06-1300 (Order No. A-69293/AJT).

Respectfully submitted,

By: _____
Aldo J. Test, No. 18,048

FLEHR HOHBACH TEST ALBRITTON &HERBERT LLP
Suite 3400, 4 Embarcadero Center
San Francisco, CA 94111-4187
Telephone: (650) 494-8700

METHOD AND APPARATUS FOR DETECTING MICROPARTICLES IN FLUID SAMPLES

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method and apparatus for detecting microparticles in fluid samples. More particularly, the present invention relates to a method and apparatus which uses a fluid delivery system and laser fluorescence detection system to detect fluorescently tagged microparticles in low concentrations in fluid samples.

Description of Related Art

Detection of microorganisms present at low concentration in fluids is critical to provide microbiological contamination answers faster to better treat patient diseases, to prevent deadly outbreaks, to better manage quality control processes in food, drink, and drug manufacturing plants, and to provide scientists with powerful and easy to use analytical research tools.

Testing methods for microorganisms such as *M. tuberculosis*, *Trichomonas vaginalis*, *Campylobacter*, *Salmonella*, *E. coli*, and *Cyclospora* include growth culture methods, PCR methods, fluorescently enhanced microscopic visualizations, ATP bioluminescence techniques, and bactometers. These methods are often slow and expensive, and have limited detection capabilities.

Testing devices include epifluorescent microscopes, fluorometers, and flow cytometers. Epifluorescent microscopes are coupled with cooled CCD high-resolution cameras to permit epifluorescent microscopic visualizations of microscopic particles. Fluorometers have limited detection capabilities, and is also not well suited when spectral differentiation in a large population of organisms is required. This is often the case when live versus dead organism differentiation is required. Flow

cytometers can be very accurate in detecting and differentiating immuno-fluorescently dead or live labeled particles. However, flow cytometers are expensive and require an experienced technician or scientist to operate it and interpret the data.

Cryptosporidium oocysts and *Giardia* cysts may be detected using an immunofluorescent assay (IFA) procedure. This method uses polyclonal antibodies to stain the cysts which then can be detected by epifluorescent microscopy. This method is extremely labor-intensive, considering the number of particles to be investigated under the epifluorescent microscope by an experienced technician. Flow cytometeters may also be used, but they are very expensive and require an experienced and well-trained technician to operate. Furthermore, flow cytometers still require microscopy confirmation of oocyst identification.

Water quality monitoring is vital for managing supplies of unpolluted water for agriculture, industry, and human consumption. Water quality monitoring may be performed using test organisms as indicators of freshwater toxicity, for example, the fathead minnow *Pimephales promelas*, the cladoceran *Ceriodaphnia dubia*, and the green alga *Selenastrum capricornutum*. Test organisms are cultured under standard conditions, and exposed for a period of time to toxicants. Comparison of survival and reproduction rates of test organisms to control organisms provides an indication of water toxicity.

Bacteria enzyme activity may be used to assess water quality by using a specially designed enzyme substrate that becomes fluorescent when cleaved. This substrate is cleaved by enzymes in the bacteria and emits fluorescence light when exposed to light of the proper wavelength. The rate of enzyme activity can be measured using a fluorometer, and provides an indirect measurement of the level of toxicant stress on the bacteria.

Zooplankton feeding behavior may also be used to assess water quality. Extensive acute toxicity studies have been performed using plankton, in general, and various species of rotifer, in particular. Rotifer feeding and reproduction rates can be used as a rapid toxicity assessment tool. The effect of a wide range of chemicals including xylene, cadmium, copper, mercury, and diazanon on the feeding and reproduction rates of the rotifer *Brachionus calyciflorus* for fresh water and *Brachionus plicatilis* for marine waters has been extensively studied. In the feeding

rate method, the rotifers are exposed for several minutes to water containing a toxicant, and then allowed to feed on fluorescently labeled beads. The rotifers are then anesthetized, washed, transferred to a microscope slide, and individually examined using a fluorescent microscope. The feeding rate is estimated by quantifying the intensity of fluorescence of ingested beads in the digestive tract of individual rotifers using an imaging technique. This method requires a trained operator, a camera, and a fluorescent microscope, which makes it slow and expensive.

What is needed are methods and apparatus for detecting microparticles such as harmful microorganisms and assessing water quality which is rapid, sensitive, reproducible, substantially automatic, and cost-effective.

SUMMARY OF THE INVENTION

The present invention is a device for detecting a fluorescent substance tagged to a microparticle. The device comprises a single capillary flow carrier system for transporting the microparticle past a selected location, a source of electromagnetic radiation for irradiating the substance tagged to the microparticle, and a detection system for measuring fluorescent light emitted from the substance at the selected location.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows a sample preparation system.

FIG. 2A shows a flow carrier system.

FIGS. 2B and 2C show detailed views of two embodiments of the capillary tube.

FIG. 3A shows one embodiment of a laser fluorescent measurement setup.

FIG. 3B shows another embodiment of a laser fluorescent measurement setup

FIG. 3C shows yet another embodiment of a laser fluorescent measurement setup

FIG. 4A shows a sample output of a digital processing unit.

FIG. 4B shows another sample output of a digital processing unit.

FIG. 5A shows the emission spectra for various fluorescent cyanide dyes used to tag microparticles.

FIG. 5B shows a flowchart of a signal processing strategy to detect a Cy5 tagged microparticle in the presence of other fluorescently labeled microparticles.

FIG. 6A shows a calibration curve obtained with water sample seeded with known amount of 2 μm fluospheres using the device of the present invention.

FIG. 6B shows a graph of the dependence of the particle concentration on the particle arrival rate distributions (Poisson statistical model).

FIG. 6C shows a graph of the evolution of the integrated detected fluorescence signal versus the SYTO™ 60 *E. coli* concentration.

FIG. 7A shows normalized bead concentrations versus feeding time according to an analytical model for three different organism concentrations.

FIG. 7B shows normalized fluosphere concentrations versus feeding time for a test sample containing 1 ppm of diazanon and a control sample, using a rotifer concentration of 500 rotifers/ml.

FIG. 7C shows normalized fluosphere concentrations versus feeding time for a test sample containing 1 ppm of diazanon and a control sample, using a rotifer concentration of 600 rotifers/ml.

FIG. 7D shows normalized fluosphere concentrations versus feeding time for a 600 rotifers/ml sample and an 1000 rotifers/ml sample.

DETAILED DESCRIPTION OF THE INVENTION

FIGURE 1 shows a sample preparation system 100. Sample preparation system 100 contains a fluid sample 102 suspected of containing microparticles 104. Fluid sample 102 may be run through a filter or trap to separate out any unwanted or macroscopic particulate matter. In order to prepare a sample to be tested, a fluorescent substance 106 is allowed to react with fluid sample 102 and microparticles 104. Fluorescent substance 106 may be one or more fluorescent stains, dyes, or reagents designed to stain, tag, or otherwise attach themselves to microparticles 104. A test sample 108 is obtained by filtering out any free remaining fluorescent substance 106 from fluid sample 102. Test sample 108 thus contains fluid sample 102 and fluorescent substance 106 attached to microparticles 104.

Fluid sample 102 may be a water sample, urine sample, blood sample, food product sample, or any other fluid to be tested. Fluid sample 102 may contain PCR-amplified samples for detection of viruses such as HIV. Microparticles 104 may be *M. tuberculosis*, *Trichomonas vaginalis*, *Campylobacter*, *Salmonella*, *E. coli*, *Cyclospora*, *Cryptosporidium* oocysts, *Giardia* cysts, or any other bacterium, virus, fungus, or microorganism that is capable of being tagged. Microparticles 104 may also be CD4 or CD8 cells for monitoring of AIDS patients. Different fluorescent substances 106 may be used with microparticles 104 to allow different types of microorganisms to be detected and distinguished from each other. For example, for bacteria, fluorescent substance 106 can be standard DNA or surface-label-reagent stains. For *Cryptosporidium* oocysts or *Giardia* cysts, fluorescent substance 106 can be a fluorescent dye conjugated with anti-*Cryptosporidium* or anti-*Giardia* antibodies, respectively. Fluorescent substance 106 may also be magnetically charged so that it can be affected by a magnetic field.

Microparticles 104 may also be microscopic beads containing a fluorescent substance 106. To measure the concentration of a toxicant in fluid sample 102, filtro-feeder microorganisms such as rotifers or zooplankton may be added to fluid sample 102 in known quantities. Such filtro-feeder microorganisms have a feeding rate which is a well-known function of toxicant concentration. After a known incubation period, microparticles 104 are added to fluid sample 102. Microparticles 104 may be fluospheres capable of being ingested by the filtro-feeder organisms, such as latex

beads containing a fluorescent dye available from Molecular Probes, Inc., Eugene, Oregon. The fluospheres may have a uniform diameter of 2 μm or have non-uniform sizes. They may have uniform spectro-photometric properties, with a maximum absorption wavelength of 624 nm, and a maximum emission wavelength of 645 nm, or have varying spectro-photometric properties. At known intervals of time, a test sample 108 is drawn from sample preparation system 100. Test sample 108 is obtained by filtering out any uningested microparticles 104 from fluid sample 102. Test sample 108 thus contains water sample 102 and organisms 104 with microparticles 104 in their digestive tracts.

FIGURE 2A shows a flow carrier system 200. Flow carrier system 200 is a fluid delivery system which introduces test sample 108 into a capillary tube 202. Capillary tube 202 may have very thin walls and excellent optical properties. Capillary tube 202 may have an internal diameter configured to admit microparticles 104 one at a time. A section of capillary tube 202 defines a test volume 204. Capillary tube 202 may be at least partially coupled to an optical table 206, which serves to hold capillary tube 202 in place. Multiple capillary tubes 202 may be arranged in parallel to obtain higher throughputs.

Flow carrier system 200 may include a pump system 216 coupled to capillary tube 202. Pump system 216 may be a syringe 208 which contains test sample 108, and injects test sample 108 through capillary tube 202 and test volume 204. After passing through capillary tube 202, sample 108 may pass into a dump 212. In this manner, microparticles 104 in test sample 102 may be passed one at a time through capillary tube 202. Pump system 216 may further include a syringe pump 214 coupled to syringe 208. Syringe pump 214 is configured for precise control of flow of test sample 108 through capillary tube 202. Pump system 216 may also be a peristaltic pump.

FIGURES 2B and 2C show detailed views of two embodiments of capillary tube 202. FIGURE 2B shows microparticles 104 flowing through capillary tube 202 towards test volume 204. FIGURE 2C shows capillary tube 202 with a magnetic element 220 positioned in a concentric fashion around capillary tube 202. Magnetic element 220 may be a continuous ring, or be comprised of one or more separate elements. Magnetic element 220 may be used in conjunction with microparticles 104

and fluorescent substance 106 which are magnetically charged. This configuration may assist in substantially focusing microparticles 104 tagged with fluorescent substance 106 to the center of capillary tube 202 as they flow through test volume 204, thus improving detection of microparticles 104.

FIGURE 3A shows a laser fluorescent measurement setup 300. A laser 302 generates a laser beam 304. Laser beam 304 may be focused through one or more lenses 306 onto test volume 204. The wavelength and beam size of laser 302 is selected according to the absorption wavelength of fluorescent substance 106 and the size of capillary tube 202.

When test sample 108 is passed through test volume 204, any fluorescent substance 106 present in test sample 108 is exposed to laser beam 302. Alternatively, a standing test sample 108 in capillary tube 202 may be moved relative to laser beam 302 to expose test sample 108. A collecting lens 310 collects and images fluorescence light emitted by fluorescent substance 106 onto a photo-multiplier 312. A set of interference filters 314 may be placed in front of photo-multiplier 312 to filter out the resonant light from the fluorescence light. A photodiode 316 may be placed on the opposite side of test chamber 308 to collect the resonant light. Output from photo-multiplier 312 may be sent to a first digital processing unit 318 to analyze fluorescence peaks. Output from photodiode 316 may be sent to a second digital processing unit 320 to analyze Mie scattering peaks.

FIGURE 3B shows another laser fluorescent measurement setup 300. In this case, the fluorescence emissions pass through a diffraction grating 313 and are imaged onto a multiple detector array 315. The focal length and aperture of collecting lens 310, the dispersion characteristics of grating 313, and the size and separation of the multiple detectors in array 315 are optimized to detect at least two or three fluorescent emission bands specific to the emission spectrum of fluorescent substance 106 as well as resonant light. A set of interference filters 317 may be used to single out the fluorescence emission of fluorescent substance 106 used to tag microparticles 104. By reading the fluorescence emission at multiple spectral locations using multiple interferential filters 317 with specific transmission characteristics, the particular fluorescent substance 106 used can be detected and distinguished. The contribution of the total fluorescence signal to each detector will provide the data needed to

differentiate the particular fluorescent substance 106 from the fluorescence emissions of non-tagged particles. Output from multiple detector array 315 may be fed to a digital processing unit 318, which processes and digitizes the multiple signals delivered by multiple detector array 315.

FIGURE 3C shows another embodiment of a laser fluorescent measurement setup 300 A plurality of lasers 302 generates a plurality of laser beams 304. Laser beams 304 may be focused through one or more lenses 306 onto test volume 204. The size of laser beam 304 may be matched to the size of capillary tube 202. The wavelengths of lasers 302 are tuned to specific absorption bands of fluorescent substance 106. This multiple laser and detection system may assist in reducing false positive and negative results associated with a single laser system.

FIGURE 4A shows a sample output from digital processing unit 318. The voltage signal coming out of photo-multiplier 312 is digitized and transferred to a computer where it can be manipulated and analyzed. The voltage signal may be digitized at a frequency of up to 3000 Hz with 8-bit precision.

Every time fluorescent substance 106 passes through test volume 204, a fluorescence peak 402 is created. A threshold value 404 may be selected according to the baseline signal level and its variance. The number of fluorescence peaks 402 detected above threshold value 404, along with the size of voltage spikes, give a measurement of the amount of fluorescent substance 106. In the case of toxicant concentration, comparing this data with the data for an uncontaminated control sample permits determination of the toxicant concentration in water sample 102.

FIGURE 4B shows another sample output from digital processing unit 318. When an microparticle 104 which has been tagged by fluorescent substance 106 passes through test volume 204, the it generates a burst of fluorescence light with a time signature 406 and spectral signature 408 The time signature 406 and spectral signature 408 is then processed by the digital signal processing unit 318 and compared with the expected time and spectral signatures of microparticle 104 and fluorescent substance 106 to be detected.

Because a wide range of particles and organisms naturally fluoresce at a wide range of wavelengths, it is crucial to spectrally differentiate an microparticle to reliably detect it. Multiple laser sources and detectors may be used in close

wavelength proximity to spectrally pinpoint the fluorescence pattern of the dye attached to the microparticle targeted for detection. The electronic signal analysis techniques can be tailored to the understanding of the pre-defined shape and spectral properties of the target microparticle prior to detection.

The use of multiple signals provides normalization and improved selectivity. Measurements at more than one fluorescence emission wavelength and/or at more than one excitation wavelength give spectral selectivity which can distinguish different dye sources. Because the dyes used for immunofluorescence commonly have relatively narrow emission peaks compared to background fluorescence sources, ratios of on-peak to off-peak signals may reliably distinguish dye-labeled particles from background events of similar absolute fluorescence.

Background particle signals are rejected through the use of electronic filtering, thereby allowing a sustained and very high sampling data rate. Electronic filtering involves the use of several detectors and is made possible by the uniqueness of a particle's light scattering signature and the presence of at least two fluorescent wavelength signatures. Based on the differential signal analysis of each of the detected log amplified signals, the capability of singling out the tagged microparticle at a data frequency rate of up to 50 kHz can be achieved.

FIGURE 5A shows emission spectra of various fluorescent cyanide dyes which may be used to tag microparticles 104: Cy5, Cy5.5, and Cy7, with maximum absorption peaks of 650 nm, 675 nm, and 743 nm, respectively. Multiple detector array 318 may be used to first record the fluorescent spectrum of the particular dye. The fluorescent spectra emitted by the tagged microparticles 104 in the sample is then compared to the recorded fluorescent spectrum of the dye. In this fashion, tagged microparticles 104 can be identified and distinguished from other fluorescently tagged microparticles 104. In addition, because the flow rate is controlled, the width of the trace signal can be considered proportional to the diameter of microparticle 104 crossing test volume 204.

FIGURE 5B shows a flowchart of a signal processing strategy to detect a Cy5 tagged microparticle 104 in the presence of other fluorescently labeled microparticles. Four detectors are used. Detector D1 is centered on the resonant laser excitation, in

this case 635 nm. Detectors D2, D3, and D4 are centered at 650 nm, 670 nm, and 690 nm, corresponding to features of the Cy5 fluorescence emission.

When a microparticle 104 is detected in test volume 204, first, the time trace of the signal detected by detector D1 is analyzed (block 502) and the signal intensity S1 and the pulse width W1 are compared with the expected time trace (Sc, Wc) generated by the passage of the particular microparticle in the test volume (block 504). If the detected signal does not meet this criteria, then the data is rejected (block 506). If the detected signal passes this first test, then the fluorescence intensity ratio of the detectors D2, D3, and D4 are analyzed (block 508). The fluorescent ratio of S2/S3 and S2/S4 are compared with the expected fluorescence ratios corresponding to the Cy5 fluorescence spectra Sca and Scb, respectively (block 510). If these two tests are positive, a microparticle is counted (block 512); if the tests are negative the data is rejected (block 514).

EXAMPLE 1

Flow carrier system 100 was calibrated using water samples with known fluosphere concentrations. A reference solution of 3×10^9 beads/ml was diluted 1000 times. Then water samples containing 0, 3, 500, 7000, 14000 and 28000 beads/ml were prepared with a 10% confidence interval using a 20 µl micropipet. These water samples were passed through the device. FIGURE 6A shows detected fluorescence peak counts versus expected counts for the calibration samples. An excellent correlation was consistently obtained.

A reference sample of 10^7 *E. coli* SYTO™ 60 DNA-stained was prepared by first killing the bacteria using a 70% isopropanol exposure for one hour and then following with three sterile washes. The *E. coli* bacteria population was then stained with a 5 µmol concentration SYTO™ 60 dye. The spectral characteristics of the SYTO™ 60 dye (Abs = 650 nm, Em = 678 nm) are very well suited for the laser-based system of the present invention.

Five graded concentration samples from 10^7 to 0 *E. coli* per ml stained were prepared using a 20 µl micropipette and 2 µm filtered de-ionized water. A 100 µl solution of each sample was drawn using a 1 ml syringe. The syringe was placed onto a syringe pump, and a 10 µl/min flow rate of the solution was injected into a 70 µm

single capillary towards the test volume. The fluorescent test volume was defined by a 20 μm focused laser beam using a 635 nm, 5 mW laser diode and a single 10 mm focal lens. The test volume was imaged onto a 3 mm x 3 mm slit using a 40x objective microscope. The photodetector signal was digitized at 3000 Hz and 8 byte dynamic range. The digitized signal was transmitted through a single serial cable onto a laptop computer. The signal was displayed on-line on a window screen using proprietary software. A time series corresponding to an injection of each sample at 10 μl/min flow rate during 30 sec was recorded. A triplicate experiment was performed for each sample, which corresponds to a 90 sec injection. By controlling the flow rate, the injection time, and the expected concentration, an expected fluorescent peak count was calculated and compared with the actual measured count. For each sample, the average peak residence time, peak intensity, and peak power (peak integral) were also computed.

The arrival process of the particle across the test volume was assumed to follow a random arrival process and therefore follow a Poisson process. The expected count number was corrected accordingly, to take into account the probability of having more than one particle arriving into the test volume during a time window equivalent to the particle transit time across the laser beam, taking into account the fact that a single detected count could be attributed to more than one particle.

FIGURE 6B represents the particle arrival rate distribution at the test volume for the organism concentration investigated. For concentrations greater than 10^5 p/ml at a 10 μl/min flow rate, a Poisson statistical correction is necessary. In particular, at a concentration of 10^7 *E. coli* per ml, there is more than one *E. coli* crossing the test volume 80% of the time.

FIGURE 6C represents a correlation between the expected concentration and the integrated detected fluorescence signal corresponding to the passage of individual stained *E. coli* through the laser test volume. The integrated signal was computed as the product of the number of detected peaks corrected by Poisson statistics, and the average peak power (V/ms). The correlation is excellent, with a 98% slope. However, when there was no *E. coli* present in the sample, an integrated background noise of 4 peaks every 30 seconds was detected. These background peaks where attributed to bubbles deflecting the beam reflection into the photo-detector slit or to

naturally fluorescent particles. The use of multiple wavelength detection arrays and a light scattering detector may eliminate these false positive counts.

EXAMPLE 2

Water quality monitoring using rotifers was performed using a capillary tube with an internal diameter of 70 μm, a narrow band, 635 nm, 3 mW diode laser with a beam diameter of 40 μm was used. Interference filters were selected to transmit 12% at 670 nm (20 nm FWM), and 10^{-6} at all other wavelengths.

Method

1. A live *B. plicatilis* rotifer culture was obtained from Aqua-Farms, Florida. These rotifers were chosen because they are easy to raise, and the influence of toxic samples on their feeding, reproduction, and death rates have been studied extensively. The average concentration of rotifers in a 100 ml vial was counted using five 20 μl samples examined under a 50x microscope. An average count of 10 rotifers per 20 μl sample was measured, or about 500 rotifers/ml.
2. Two samples of 8 ml each were used to make the feeding rate measurements, a reference sample and test sample. These two vials were filled with the 500 rotifers/ml reference solution.
3. A 2000 ppm diazanon solution was prepared using the rotifer medium solution, so as to maintain water quality parameters such as pH, O_2, alkalinity, salinity, and temperature as constant as possible. A 20 μl amount of the diazanon solution was added to test sample.
4. After a 5 minute incubation, 20 μl of a 40 x 10^6 beads/ml solution of crimson fluospheres was added to both the reference and test samples. The time was noted as t = 0.
5. Using two identical syringes connected with a luer union to a 100 μm piece of nylon tubing terminated with a 20 μm plankton filter, a 50 μl sample was extracted from the reference and test samples. In both cases, the organisms were filtered out from the bead solution.

6. The reference and test samples were successively passed through the test chamber using the syringe pump at a flow rate of 15 µl/min. The data from data acquisition were stored for later analysis.

7. Steps 5 and 6 were repeated at t = 5, 10, 15, and 20 minutes.

Data Analysis

Each data file was retrieved, using TOXANA™, a time series data analysis software program. This program allows visualization of the digitized photo-multiplier trace signal on a 0-5 V scale for each data file. An assessment of the signal baseline mean and variance values (where no peak is detected) was made for each file. From this measurement, a peak detection threshold, T_d, was computed as follows:

$$T_d = mean + 2\sqrt{variance}$$

The number of peaks with an intensity above T_d were calculated for each file, as well as the average peak intensity, the average peak width, and the average peak area.

Analytical Model

The clearance volume V_{Cl} for an organism with an average motility Ω and clearance diameter d can be assumed to be:

(Eq. 1) $$V_{Cl} = \Omega \cdot \pi \cdot \frac{d^2}{4}$$

The number of beads present in the clearance volume per unit of time is equal to:

(Eq. 2) $$\frac{dN_b}{dt} = C_b(t) \cdot V_{Cl}$$

Assume that a volume V contains organisms with an average motility Ω. In this volume the concentration of food particles or beads is $C_b(t)$.

The change in bead concentration varies per unit of time:

(Eq. 3) $$\dot{C}_b = \frac{d(C_b(t))}{dt} = -\frac{dN_b(t)}{dt} \cdot \frac{N_0}{V}$$

By substituting (1) and (2) into (3), the rate at which the bead concentration varies with time is governed by the differential equation:

(Eq. 4) $$\frac{d(C_b(t))}{dt} = -C_b(t) \cdot \pi \cdot \frac{d^2}{4} \cdot \Omega \cdot \frac{N_0}{4}$$

Define the constant K as:

(Eq. 5) $$K = \pi \cdot \frac{d^2}{4} \cdot \Omega \cdot \frac{N_0}{V}$$

Then 1/K is a time constant which reflects the rate at which the bead concentration decreases. Then (5) becomes:

(Eq. 6) $$\frac{d(C_b(t))}{dt} = -K \cdot C_b(t)$$

Integrating (6) gives:

(Eq. 7) $$C_b(t) = \lambda \cdot e^{-Kt}$$

When t = 0 and $C_N(t = 0) = C_{B0}$, (7) becomes:

(Eq. 8) $$C_b(t) = C_{B0} \cdot e^{-Kt}$$

The feeding rate is defined as the number of beads ingested per organism and per unit of time. It can be expressed by:

(Eq. 9) $$FR = \frac{dN_b}{dt} \cdot \frac{1}{N_0} = \frac{d(C_b)}{dt} \cdot \frac{V}{N_0}$$

Combining (8) and (9), F can be expressed as follows:

(Eq. 10) $$FR = K \cdot C_b(t) \cdot \frac{V}{N_0}$$

Assuming that $d(C_b)/dt$ is small compared with $C_b(t)$, F becomes a constant which can be expressed as:

(Eq. 11) $$FR = K \cdot C_b \cdot \frac{V}{N_0}$$

FIGURE 7A shows normalized bead concentrations versus feeding time according to the model given in Eq. 8 for three different organism concentrations: 100 rotifers/ml, 250 rotifers/ml, and 1000 rotifers/ml. The analytical value used to model the feeding rate (FR) in this case is 2.7 beads/min.

This value was computed based on the following assumptions: the organism is a rotifer and its clearance rate is proportional to a 150 μm diameter section with a motility of 15 cm/s. It is important to note that the sensitivity of the technique strongly depends strongly on the organism concentration.

Results

FIGURE 7B shows normalized fluosphere concentrations versus feeding time for a test sample containing 1 ppm of diazanon and a control sample. To compare the experimental results to the model, a concentration of 500 rotifers/ml was used.

For the control sample the correlation between model and experiment is excellent for the first 600 seconds. In this region, the average feeding rate is 29×10^{-3} beads/sec. The departure of experiment from the model after 900 seconds can be attributed to two factors. First, the model assumes that the variation of C_b remains small compared to C_b and therefore the feeding rate is a constant. In fact, the feeding rate depends on the food concentration, which after 900 seconds has dropped by 50%. This variation cannot be neglected. Second, the rotifers have an average digestion transit time of 1200 seconds. Since the fluospheres are not metabolized by the rotifers, they are ejected back into the sample by the rotifer after 1000 seconds, which may contribute to an increase in bead concentration.

For the test sample the concentration of fluospheres decreases slightly with a slope corresponding to an ingestion rate per organism of 10^{-3} beads/sec. Here, the feeding rate was reduced by a factor of 30 from exposure to 1 ppm of diazanon.

FIGURE 7C shows normalized fluosphere concentrations versus feeding time for a test sample containing 1 ppm of diazanon and a control sample. The concentration of organisms is now 600 rotifers/ml.

Again, for the control sample, the agreement between model and experiment is excellent for the first 600 seconds. The feeding rate is now 3 beads/min per organism, compared well to the 1.7 beads/min per organism value obtained earlier. For the test sample, the concentration of beads remains almost unchanged with time and indicates a feeding rate of less than 0.05 beads/min. This measurement is consistent with previous experiments.

FIGURE 7D shows normalized fluosphere concentrations versus feeding time for a 600 rotifers/ml sample and an 1000 rotifers/ml sample. The two samples are exposed to concentrated 2 μm fluospheres for 1700 seconds. The fluosphere concentrations are monitored continuously and the normalized concentrations are reported and compared to the clearance rate model described earlier. The agreement between model and experiment is optimum for an average feeding rate per organism equal to 4.8 beads/min.

To express feeding rate in terms of mass, the following equation may be used:

(Eq. 12) $$\dot{M} = FR \cdot \rho \cdot V_{fluo}$$

where FR, ρ, and V_{fluo} are the average feeding rate per individual rotifer, the fluosphere density, and the individual fluosphere volume, respectively. Here, for a feeding rate of 4.8 beads/min and spherical fluospheres with a density of 1.055 g/ml and a diameter of 2 μm, M-dot = 21×10^{-12} g/min per organism.

* * * * *

The foregoing description of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise forms disclosed. Obviously, many modifications and variations will be apparent to practitioners skilled in this art. It is intended that the scope of the invention be defined by the following claims and their equivalents.

What is claimed is:

CLAIMS

1. A device for detecting a microparticle in a fluid, the microparticle being tagged with a fluorescent substance, the fluorescent substance emitting fluorescent light when exposed to electromagnetic radiation, the device comprising:

a capillary chamber;

a fluid delivery system coupled to the capillary chamber, the fluid delivery system capable of introducing the microparticle and the fluid into the capillary chamber;

a source of electromagnetic radiation positioned in proximity to the capillary chamber to expose the fluorescent substance to electromagnetic radiation; and

a detection device configured to measure fluorescent light emitted from the fluorescent substance when the microparticle is in the capillary chamber.

2. The device of claim 1, wherein the microparticle is a microorganism.

3. The device of claim 1, wherein the microparticle is a bacterium, virus, or parasite.

4. The device of claim 1, wherein the microparticle is a CD4 cell.

5. The device of claim 1, wherein the microparticle is a fluosphere.

6. The device of claim 5, wherein the fluosphere has been ingested by a filtro-feeder.

7. The device of claim 6, wherein the filtro-feeder has a feeding rate sensitive to a toxicant level in the fluid sample.

8. The device of claim 1, wherein the fluorescent substance is a dye-conjugated antibody.

9. The device of claim 1, wherein the fluorescent substance is a DNA stain.

10. The device of claim 1, wherein the fluorescent substance has a magnetic charge.

11. The device of claim 10, further comprising:

a magnetic element positioned in a surrounding relationship to the capillary, the magnetic element having a magnetic charge which repels the fluorescent substance.

12. The device of claim 1, wherein the fluid delivery system is a syringe coupled to a syringe pump.

13. The device of claim 1, wherein the fluid delivery system is a peristaltic pump.

14. The device of claim 1, wherein the source of electromagnetic radiation is at least one laser.

15. The device of claim 1, wherein the detection device is an array of detectors.

16. A device for detecting a fluorescent substance tagged to a microparticle, comprising:

a single capillary flow carrier system for transporting the microparticle past a selected location;

a source of electromagnetic radiation for irradiating the substance tagged to the microparticle; and

a detection system for measuring fluorescent light emitted from the substance at the selected location.

17. The device of claim 16, wherein the source of electromagnetic radiation comprises a source of light.

18. The device of claim 17, wherein the source of light comprises a laser.

19. The device of claim 16, wherein a plurality of microparticles are individually transported past the selected location at a substantially uniform velocity.

20. The device of claim 16, wherein the microparticle is an organism.

21. The device of claim 16, wherein the microparticle is a fluosphere.

22. The device of claim 21, wherein the fluosphere is ingested by a filtro-feeder.

23. The device of claim 22, further comprising a device for exposing the filtro-feeder to a toxic substance.

24. The device of claim 23, wherein the fluorescent substance is ingested by the filtro-feeder, and exposure of the filtro-feeder to the toxic substance affects the rate of ingestion of the fluorescent substance by the filtro-feeder.

25. The device of claim 24, further comprising means for calculating the ingestion rate as a function of the amount of fluorescent light emitted from the fluorescent substance at the selected location.

26. The device of claim 16, wherein the fluorescent substance has a magnetic charge.

27. The device of claim 26, further comprising:

a magnetic element positioned in a surrounding relationship adjacent to the selected location, the magnetic element having a magnetic charge which repels the fluorescent substance.

28. A method for detecting a microparticle tagged with a fluorescent substance, comprising:

transporting the microparticle to a selected location;

irradiating the fluorescent substance tagged to the microparticle; and

measuring fluorescent light emitted from the fluorescent substance at the selected location.

29. The device of claim 28, wherein the microparticle is a bacterium.

30. The device of claim 28, wherein the microparticle is a virus.

31. The device of claim 28, wherein the microparticle is a parasitic cyst.

32. The device of claim 28, wherein the microparticle is a CD4 cell.

33. The device of claim 28, wherein the microparticle is a fluosphere.

34. The device of claim 33, wherein the fluosphere has been ingested by a filtro-feeder.

35. The device of claim 34, wherein the filtro-feeder has a feeding rate sensitive to a toxicant level in the fluid sample.

36. The device of claim 28, wherein the fluorescent substance is a dye-conjugated antibody.

37. The device of claim 28, wherein the fluorescent substance has a magnetic charge.

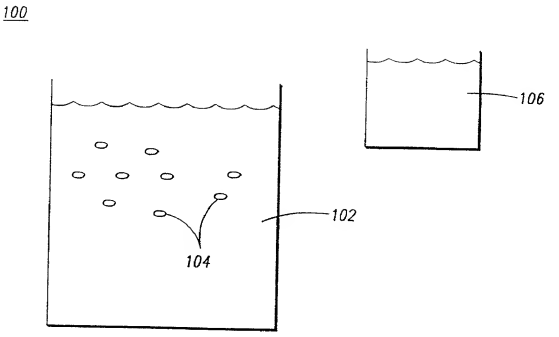

FIG. – 1

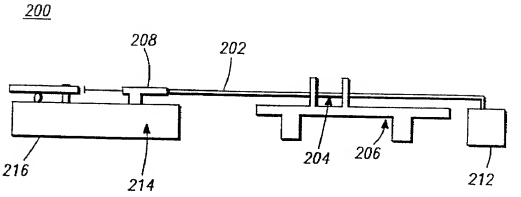

FIG. – 2A

FIG.-2B

FIG.-2C

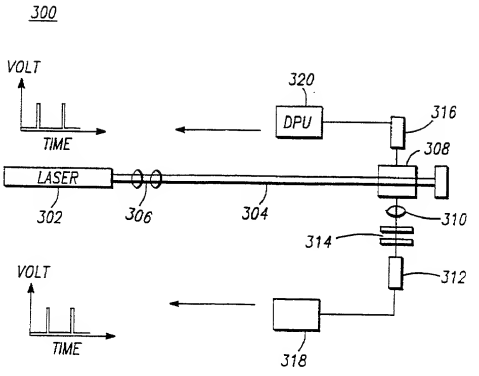
300
VOLT
TIME
320
DPU
316
308
LASER
302
306
304
310
314
312
VOLT
TIME
318

FIG.—3A

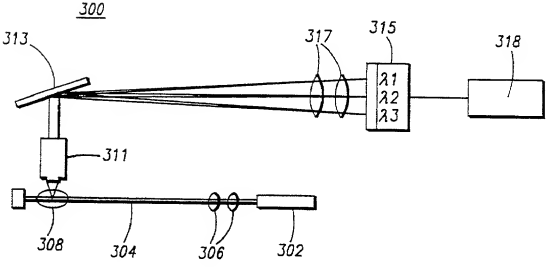
300
313
317
315
318
λ1
λ2
λ3
311
308
304
306
302

FIG.—3B

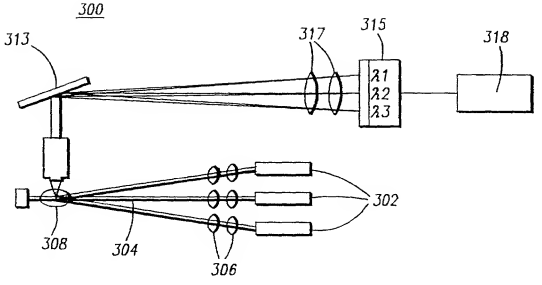
300
313
317
315
318
λ1
λ2
λ3
302
308
304
306

FIG.—3C

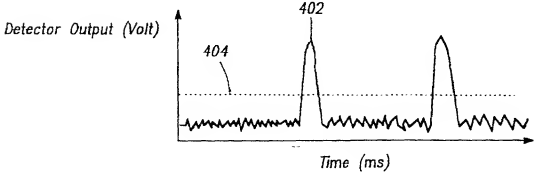
Detector Output (Volt)
402
404
Time (ms)

FIG.—4A

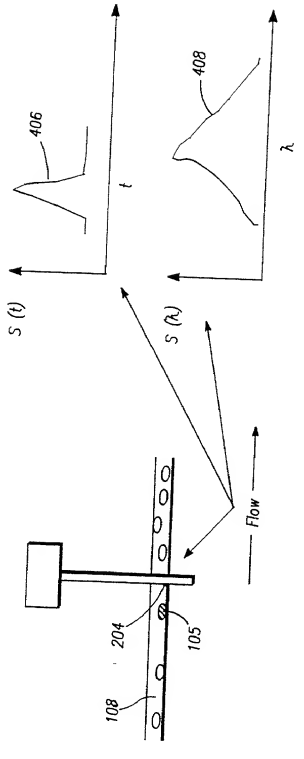

FIG. -4B

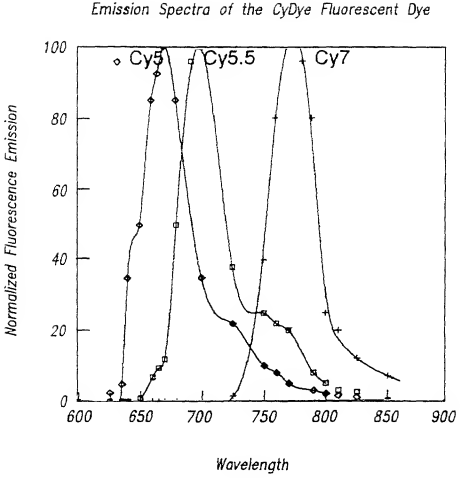
Emission Spectra of the CyDye Fluorescent Dye
Cy5
Cy5.5
Cy7
Normalized Fluorescence Emission
100
80
60
40
20
0
600
650
700
750
800
850
900
Wavelength

FIG.—5A

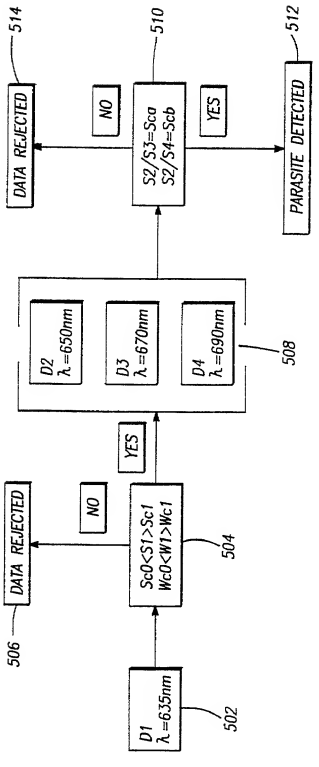
D1
λ=635nm
502
ScO<S1>Sc1
WcO<W1>Wc1
504
DATA REJECTED
506
NO
YES
D2
λ=650nm
D3
λ=670nm
D4
λ=690nm
508
S2/S3=Sca
S2/S4=Scb
510
DATA REJECTED
514
NO
YES
PARASITE DETECTED
512

FIG. —5B

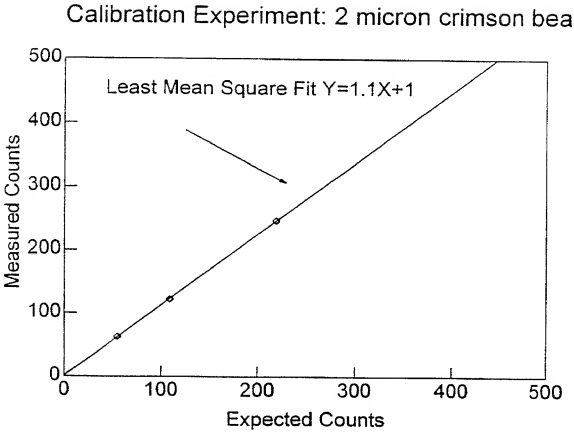
Calibration Experiment: 2 micron crimson beads
Least Mean Square Fit Y=1.1X+1
Measured Counts
Expected Counts
0
100
200
300
400
500

FIG. —6A

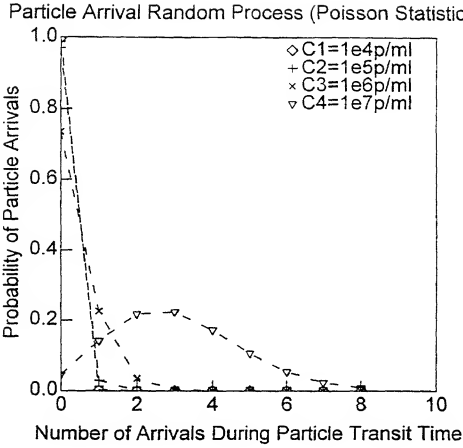
Particle Arrival Random Process (Poisson Statistics)
◇C1=1e4p/ml
+C2=1e5p/ml
×C3=1e6p/ml
▽C4=1e7p/ml
Probability of Particle Arrivals
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
Number of Arrivals During Particle Transit Time

FIG.—6B

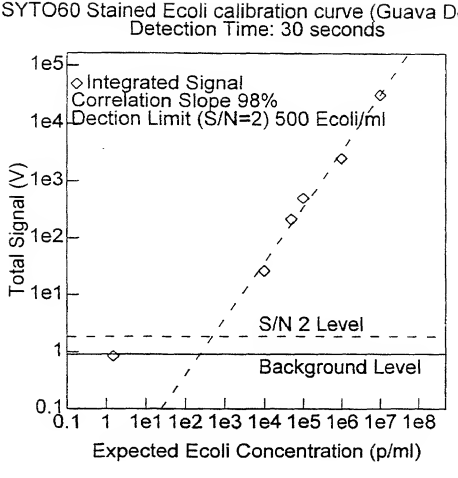
SYTO60 Stained Ecoli calibration curve (Guava Device)
Detection Time: 30 seconds
◇Integrated Signal
Correlation Slope 98%
Dection Limit (S/N=2) 500 Ecoli/ml
S/N 2 Level
Background Level
Total Signal (V)
1e5
1e4
1e3
1e2
1e1
1
0.1
0.1 1 1e1 1e2 1e3 1e4 1e5 1e6 1e7 1e8
Expected Ecoli Concentration (p/ml)

FIG.—6C

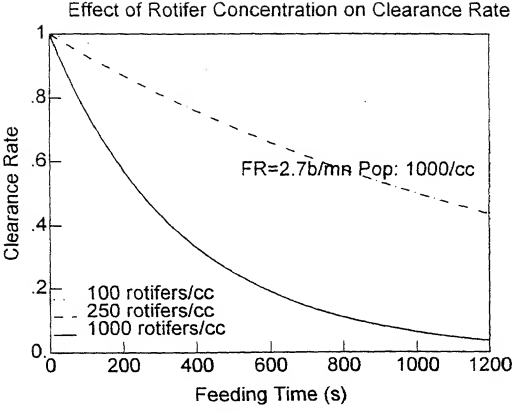
Effect of Rotifer Concentration on Clearance Rate
Clearance Rate
1
.8
.6
.4
.2
0
0
200
400
600
800
1000
1200
Feeding Time (s)
FR=2.7b/mR Pop: 1000/cc
100 rotifers/cc
250 rotifers/cc
1000 rotifers/cc

FIG. –7A

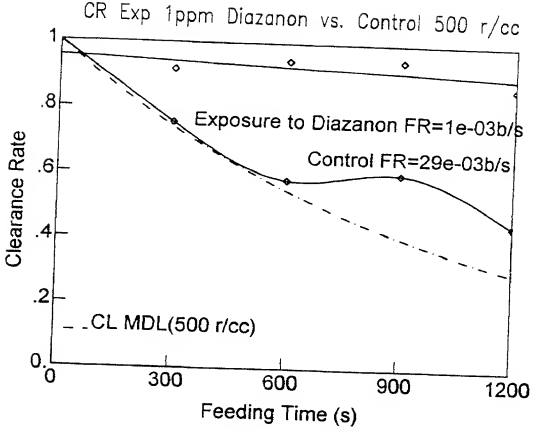
CR Exp 1ppm Diazanon vs. Control 500 r/cc
Clearance Rate
1
.8
.6
.4
.2
0
0
300
600
900
1200
Feeding Time (s)
Exposure to Diazanon FR=1e-03b/s
Control FR=29e-03b/s
CL MDL(500 r/cc)

FIG. – 7B

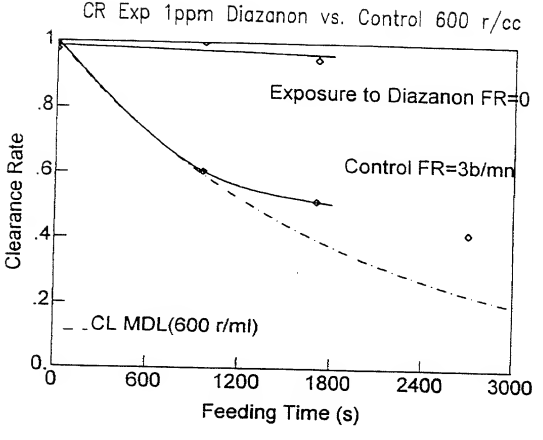
CR Exp 1ppm Diazanon vs. Control 600 r/cc
Exposure to Diazanon FR=0
Control FR=3b/mn
CL MDL(600 r/ml)
Clearance Rate
1
.8
.6
.4
.2
0
0
600
1200
1800
2400
3000
Feeding Time (s)

FIG.—7C

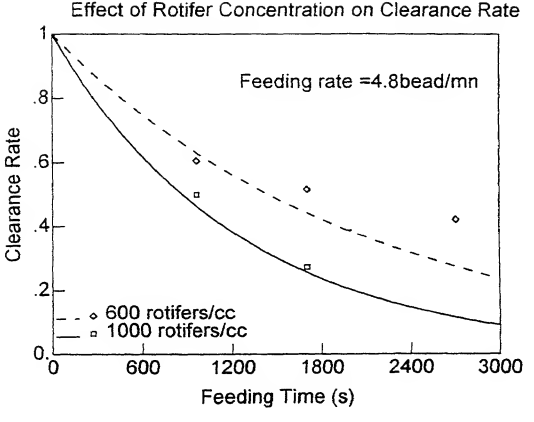
Effect of Rotifer Concentration on Clearance Rate
Feeding rate =4.8bead/mn
Clearance Rate
1
.8
.6
.4
.2
0
600 rotifers/cc
1000 rotifers/cc
0
600
1200
1800
2400
3000
Feeding Time (s)

FIG.—7D

Please type a plus sign (+) inside this box → +

PTO/SB/01 (12-97)
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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing **OR** ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	A-69293/AJT
First Named Inventor	Philippe J. Goix
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Method and Apparatus for Detecting Microparticles in Fluid Samples

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 06/09/1998 as United States Application Number or PCT International Application Number US98/11958 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/049,212	06/09/1997	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
PCT/US98/11958	06/09/1998	WO 98/57152

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*
OR
☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Aldo J. Test	18,048	Maria S. Swiatek	37,244

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] *OR* ☒ Correspondence address below

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Country	US	**Telephone**	650-494-8700	**Fax**	650-494-8771

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Philippe J.	Goix

Inventor's Signature						**Date**	6-29-00
Residence: City	Oakland	**State**	CA	**Country**	US	**Citizenship**	France
Post Office Address	5612 Florence Terrace						
Post Office Address							
City	Oakland	**State**	CA	**ZIP**	94611	**Country**	US

☐ Additional inventors are being named on the ____ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto